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Resurrection of *Petunia occidentalis* R. E. Fr. (Solanaceae) Inferred from a Cross Compatibility Study

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In order to obtain additional taxonomic information about infraspecific taxa of *Petunia integrifolia*, we examined reciprocal cross compatibility between combinations of var. *integrifolia*, var. *depauperata*, subsp. *inflata*, subsp. *occidentalis*, as well as *P. axillaris*. Subsp. *occidentalis* completely failed to set capsule when it was used as the pollen parent. Similarly, the crossings in the opposite direction were rarely successful, and the number of hybrid plants obtained was limited. In contrast, all other taxa, including *P. axillaris*, could be crossed reciprocally with a higher rate of capsule set. The pollen fertility of the subsp. *occidentalis* hybrid was inferior to that of all the other hybrids. Based on the results of a previous study (Ando et al. 1995) and those of the present one, we treated subsp. *occidentalis* as an independent species herein.

Petunia integrifolia (Hook.) Schinz. & Thell., one of the historical parents of garden petunias, is regarded as comprising four infraspecific taxa: subsp. *integrifolia* (Hook.) Wijsman with var. *depauperata* (R. E. Fr.) L. B. Sm. & Downs, subsp. *inflata* (R. E. Fr.) Wijsman, and subsp. *occidentalis* (R. E. Fr.) Wijsman. Initially, Fries (1911) regarded these three subspecies as independent species, and the variety *depauperata* as a subspecies of *P. integrifolia*. Thereafter, Wijsman (1982) treated *P. inflata* R. E. Fr. and *P. occidentalis* R. E. Fr. as subspecies of *P. integrifolia*, because he maintained that the pedicel condition during fruiting (inflexed or deflexed), an important character differentiating these subspecies, was variable and suggested overlapping between the subspecies. In a previous paper reporting the results of multivariate analysis of morphological characters of reproductive organs (Ando et al. 1995), however, we could not confirm the overlapping of the characters, including the pedicel condition, between any infraspecific taxa of *P. integrifolia*. Instead, we revealed clear separation in the morphological characters, rather supporting the older treatment of Fries (1911).

In order to obtain additional information about the taxonomic relationships in the infraspecific taxa of *P. integrifolia*, we checked their reciprocal cross compatibility as well as that of *P. axillaris* (Lam.) Britton, Sterns & Poggenb., another parental species of the garden petunia.

Materials and Methods

1) Plant materials

Seeds of respective infraspecific taxa of *Petunia integrifolia* as well as those of *P. axillaris* were collected from the voucher specimens listed in the appendix. Seeds were sown in January, and the plants were grown in a greenhouse by the standard horticultural procedure for garden petunias.

2) Crossing

Emasculation was made for the flowers of seed parents one day before anthesis, and fresh pollen grains were used for pollination on the next day.

3) Confirmation of hybridization

In order to confirm the hybridization, corolla size and color of the progenies were compared with those of the parents when *P. axillaris* was used as a parent. For the remaining combination of crossings, we compared 33 morphological characters of reproductive organs analyzed in a previous paper (Ando et al. 1995), and five colorimetric values of corolla lobes measured by a colorimetric meter (Minolta CR-300) according to Hunter's L, a, b system (Hunter 1958) (Table 1).

4) Pollen fertility

Pollen fertility of given *Petunia* taxa and their hybrids was determined by the stainability of the pollen grains with 3% aceto-carmine.

Results

1) Confirmation of hybridization

Progenies made by the reciprocal crossing of *P. axillaris* (flower white, largest in the genus) possessed corollas of pale violet and of a size as large as that of *P. axillaris*, and were safely regarded as hybrid.

Morphological features of the progenies obtained by the crossing between the remaining taxa are summarized in Table 1. Abbreviations of the characters are identical to those used in a previous paper (Ando et al. 1995). Characters X_{20} (length of pedicel), and X_{28} (number of node bearing the first flower, main stem) were not measured in this study, because these characters had not been found to be significantly different between any infraspecific taxa of *P. integrifolia* in a previous study (Ando et al. 1995). The difference in character X_{29} (number of node bearing the first flower, side stem) between them was not significant this time.

Index characters ensuring the hybridization were different depending on the taxon concerned (Table 1); solid circles (●) indicate the cases that the character values of the progeny were intermediate, differing significantly from those of the both parents; solid triangles indicate the cases that the character values were significantly larger (▲) or smaller (▼) than those of either parent.

For the progenies of subsp. *occidentalis*, longevity of flowers was also a good index of the hybridization because the flowers of the progenies lasted for several days unlike those of subsp. *occidentalis*, which faded on the same day of anthesis. When var. *depauperata* (stem prostrating) was used as the pollen parent, the characteristic prostrating stem was a reliable index to ensure the hybridization, too.

It is worthy to note that angles of the pedicel of the progenies during fruiting were intermediate between those of the parents (Table 1), since it had been considered as the most important character differentiating the given taxa (Ando et al. 1995, Fries 1911, Wijsman 1982).

2) Cross compatibility

As shown in Table 2, all crossings between different individuals of the same taxon showed the rate of capsule set of nearly 100% (from 96.2% to 100%), except for those of var. *depauperata* (77.8%).

All combinations of reciprocal crossing among var. *integrifolia*, var. *depauperata*, and subsp. *inflata* showed high rates of capsule set (from 86.7 to 100%). When subsp. *occidentalis* was used as the pollen parent, however, all

Table 1. Comparison of floral characters between parents and progenies obtained by reciprocal crossing of infraspecific taxa of *Petunia integrifolia*

Characters measured	The symbols are the same as those used in Ando et al. (1995)	Seed (upper row) and pollen (lower) parent								
		OCC INT	OCC INF	OCC DEP	INT INF	INF INT	INF DEP	DEP INF	INT DEP	DEP INT
X_1	distance between anthers of long and medium stamens	●	○	●	▲	▲	△	△	○	△
X_2	distance between anthers of medium and short stamens	△	△	▲	○	○	▲	▲	○	○
X_3	distance between two anthers of long stamens	●	○	△	△	▽	△	△	▽	○
X_4	distance between two anthers of medium stamens	○	○	○	△	▽	▽	▽	▽	○
X_5	length of long stamens	▲	○	▲	○	●	○	△	○	△
X_6	length of basal part of filament affixed to corolla-tube	●	●	○	●	●	○	○	○	○
X_7	ratio X_6/X_5	●	●	●	●	○	○	○	△	○
X_8	length of pistil plus ovary	▲	●	▲	●	●	○	△	○	○
X_9	length along vertical axis of stigma	▲	▲	▲	○	△	○	○	△	▲
X_{10}	length along horizontal axis of stigma	●	△	▲	○	○	△	△	○	●
X_{11}	ratio X_9/X_{10}	△	●	▲	○	△	○	▽	○	○
X_{12}	length of upper calyx-lobe	△	▲	○	△	▲	○	○	○	○
X_{13}	width of upper calyx-lobe at the central part	○	△	▽	△	△	△	△	▽	△
X_{14}	length of calyx-tube	△	○	△	○	△	○	○	○	△
X_{15}	ratio X_{12}/X_5	△	△	○	△	△	○	●	○	○
X_{16}	length along long axis of ovary	●	●	●	△	○	○	○	▽	▽
X_{17}	length along horizontal axis of ovary	○	●	○	△	○	○	○	△	○
X_{18}	length along vertical axis of ovary	●	○	○	△	△	△	○	△	○
X_{19}	ratio X_6/X_{16}	△	●	▲	○	○	○	△	△	△
X_{21}	length of corolla-tube	▲	▲	▲	●	●	△	▲	●	●
X_{22}	ratio X_8/X_{21}	▽	▼	▼	○	○	●	●	○	○
X_{23}	ratio X_{13}/X_8	△	○	○	△	△	○	●	○	○
X_{24}	diameter of corolla-tube	●	▲	▲	○	●	▲	△	○	○
X_{25}	length of corolla	●	▲	▲	●	○	△	▲	○	○
X_{26}	width of corolla-limb	●	△	▲	△	▲	○	△	●	●
X_{27}	ratio X_{25}/X_{26}	●	●	●	●	●	○	○	△	△
X_{30}	length of capsule	▼	▼	▼	▽	▽	▼	○	▽	▽
X_{31}	width of capsule	▼	▼	▼	▽	▽	▽	▲	▽	▽
X_{32}	ratio X_{30}/X_{31}	●	●	○	○	○	▽	▼	▽	○
X_{33}	angle of pedicel to stem during fruiting	●	●	●	●	●	●	●	○	○
	Hunter's L value	○	●	●	●	●	○	●	△	▽
	a value	△	▲	△	△	△	○	△	▽	△
	b value	▽	▼	▽	▼	○	○	○	○	▲
	a / b	●	●	●	△	○	○	○	△	▼
	$\sqrt{a^2 + b^2}$	△	▲	▲	▲	△	○	△	▽	△

INT: *P. integrifolia* subsp. *integrifolia* var. *integrifolia*.
DEP: *P. integrifolia* subsp. *integrifolia* var. *depauperata*.
INF: *P. integrifolia* subsp. *inflata*.
OCC: *P. integrifolia* subsp. *occidentalis*.
▲△ : average value of the progeny larger than those of both parents.
●○ : average value of the progeny intermediate between those of the parents.
▼▽ : average value of the progeny smaller than those of both parents.
▲●▼ : significant at 5% level by t-test.
△○▽ : not significant.

Table 2. Cross compatibility between various combinations of infraspecific taxa of *Petunia integrifolia* and *P. axillaris*

Seed parent		Pollen parent		No. set / No. crossing set ratio (%)		
		INT	DEP	INF	OCC	AX
Petunia integrifolia subsp. *integrifolia* var. *integrifolia*	INT	25/26 96.2	14/15 93.3	13/15 86.7	0/36 0.0	4/42 9.5
var. *depauperata*	DEP	14/15 93.3	21/27 77.8	14/15 93.3	0/34 0.0	2/49 4.1
subsp. *inflata*	INF	14/15 93.3	15/15 100.0	39/39 100.0	0/33 0.0	6/35 17.1
subsp. *occidentalis*	OCC	6/22 27.3	14/24 58.3	3/22 13.6	37/37 100.0	5/33 15.2
Petunia axillaris	AX	6/15 40.0	8/15 53.3	2/38 5.3	0/20 0.0	23/23 100.0

crossings with the other infraspecific taxa of *P. integrifolia*, as well as the crossing with *P. axillaris*, completely failed to set capsule. In contrast, when subsp. *occidentalis* was used as the seed parent, it could be crossed with the other infraspecific taxa of *P. integrifolia*, even though the rates of capsule set were low (from 13.6 to 58.3%) (Table 2). These features of cross compatibility among infraspecific taxa of *P. integrifolia* as well as *P. axillaris* are illustrated in Fig. 1.

Except for the crossing with subsp. *occidentalis*, a large number of hybrid plants (more than 50 plants) were obtained from one capsule. The numbers of hybrid plants obtained by the crossing of subsp. *occidentalis*, however, were very limited: only one plant per capsule was obtained for subsp. *occidentalis* × subsp. *inflata* and for subsp. *occidentalis* × *P. axillaris*, and less than 10 hybrid plants were obtained from one capsule for subsp. *occidentalis* × subsp. *integrifolia* (an average of 3.3 plants per capsule) and for subsp. *occidentalis* × var. *depauperata* (9.3 plants per capsule).

3) Pollen fertility of the hybrid

As shown in Table 3, five taxa of *Petunia* used in this study exhibited pollen fertility higher than 90% (from 92.8 to 98.8%). Among the hybrids thus obtained, those made by reciprocal crossings between var. *integrifolia* and subsp. *inflata* showed the highest average value for pollen fertility (88.9 and 84.9%), followed by those between var. *integrifolia* and var. *depauperata* (84.6 and 78.2%), and those between subsp. *inflata* and var. *depauperata* (69.2 and 73.2%). All hybrids of subsp. *occidentalis* crossed with the other infraspecific taxa of *P. integrifolia*, however, showed lower values for this parameter (from

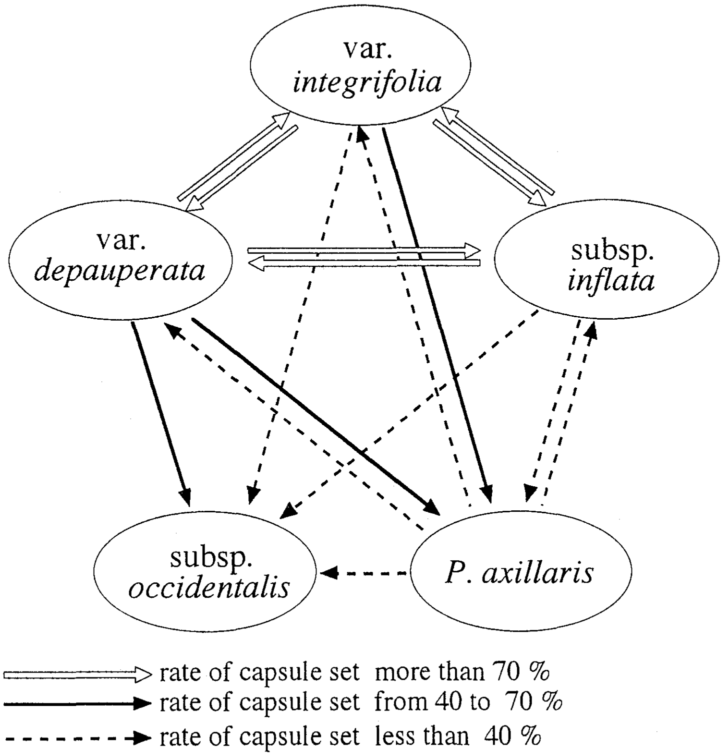
var. integrifolia
var. depauperata
subsp. inflata
subsp. occidentalis
P. axillaris
rate of capsule set more than 70 %
rate of capsule set from 40 to 70 %
rate of capsule set less than 40 %

Fig. 1. Reciprocal cross compatibility between combinations of four infraspecific taxa of *Petunia integrifolia* and *P. axillaris*, as illustrated by the rate of capsule set.

Table 3. Pollen fertility of the hybrids obtained by the crossing between various combinations of infraspecific taxa of *Petunia integrifolia* and *P. axillaris*

Seed parent		Pollen fertility (%) Pollen parent				
		INT	DEP	INF	OCC	AX
Petunia integrifolia subsp. *integrifolia* var. *integrifolia*	INT	95.5	84.6	88.9	–	71.4
var. *depauperata*	DEP	78.2	92.8	73.2	–	51.4
subsp. *inflata*	INF	84.9	69.2	98.8	–	70.2
subsp. *occidentalis*	OCC	40.5	38.8	40.1	96.9	69.5
Petunia axillaris	AX	69.5	56.9	66.6	–	97.0

38.8 to 40.5%).

Discussion and Conclusion

Even though index characters confirming the hybridization were variable among respective combinations of crossing (Table 1), we could safely regard all progenies thus obtained as hybrids. The lower pollen fertility of the progenies (Table 3) may also ensure successful crossing.

It is reasonable to say that var. *integrifolia*, var. *depauperata*, and subsp. *inflata* were freely crossable between one another (Table 2, Fig. 1). In contrast, the hybrid of subsp. *occidentalis* was obtainable only when it served as the seed parent (Table 2, Fig. 1), as the crossing in the opposite direction yielded no hybrids. Subsp. *occidentalis* is known to be distinguishable from the other infraspecific taxa of *P. integrifolia* by the nature of its unilateral incompatibility (Lewis and Crowe 1958). In addition, the rates of capsule set were exceptionally low (Table 2, Fig. 1), and the number of hybrid plants obtained was very limited (Table 2) when subsp. *occidentalis* was crossed with the other infraspecific taxa.

In the previous paper (Watanabe et al. 1996), six cultivars of *Petunia* crossed with subsp. *occidentalis* completely failed to set capsule, even though the cultivars × other infraspecific taxa of *P. integrifolia* did so almost completely.

All progenies made by the crossing among var. *integrifolia*, var. *depauperata*, and subsp. *inflata* exhibited higher pollen viability (from 69.2 to 88.9%) than those of subsp. *occidentalis* (from 38.8 to 40.5%) (Table 3).

In addition to the results published previously (Ando et al. 1995), showing clear separation of morphological characters among infraspecific taxa of *P. integrifolia*, and characteristics of subsp. *occidentalis* rather unique to the genus, the evidence mentioned above strongly suggests that taxon *occidentalis* is not an infraspecific taxon of *P. integrifolia*, but an independent species, as Fries (1911) treated it. Whereas, subsp. *inflata* is evidently closely related to subsp. *integrifolia*, and could be placed as a subspecies of *P. integrifolia*.

It is interesting to note the pollen fertility of subsp. *occidentalis* × *P. axillaris* hybrids (69.5%), because it was superior to that of any hybrids of subsp. *occidentalis* crossed with infraspecific taxa of *P. integrifolia* (from 38.8 to 40.5%). It is also noteworthy that the pollen fertility of subsp. *occidentalis* crossed with the infraspecific taxa of *P. integrifolia* (from 38.8 to 40.5%) was inferior to that obtained from *P. axillaris* crossed by the corresponding infraspecific taxa of *P. integrifolia* (from 56.9 to 69.5%). These facts may suggest that subsp. *occidentalis* is more closely related to *P. axillaris* than to subsp. *integrifolia* or subsp. *inflata*.

Enumeration

Petunia occidentalis R. E. Fr. in Kungl. Svensk. Vet. Akad. Handl. **46(5)**: 1–72, Tab. 2, 5 (1911); Á. L. Cabrera in Flora de la provincia de Jujuy. 324. f. 135 (1983).

Petunia integrifolia (Hook.) Schinz. & Thell. subsp. *occidentalis* (R. E. Fr.) Wijsman, Acta Bot. Neerl. **31**: 477 (1982); T. Ando et al., Tech. Bull. Fac. Hort. Chiba Univ. **45**: 17 (1992); T. Ando and G. Hashimoto, Bot. J. Lin. Soc. **111**: 265 (1993); T. Ando et al., J. Jpn. Bot. **70(4)**: 205, f. 3, 4, 5 (1995); T. Ando and G. Hashimoto, Brittonia **48(2)**: 217 (1996); H. Watanabe et al., J. Japan. Soc. Hort. Sci. **65(3)**: 625 (1996); H. Watanabe et al., J. Japan. Soc. Hort. Sci. **66(3/4)**: 607 (1997).

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Soc. America **48**: 985–995.

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Watanabe H., Ando T., Iida S., Suzuki A., Buto K., Tsukamoto T., Hashimoto G. and Marchesi E. 1996. Cross compatibility of *Petunia* cultivars and *P. axillaris* with native taxa of *Petunia* in relation to their chromosome number. J. Japan. Soc. Hort. Sci. **65(3)**: 625–634.

Wijsman J. R. 1982. On the inter-relationships of certain species of *Petunia* I. Taxonomic notes on the parental species of *Petunia hybrida.* Acta Bot. Neerl. **31**: 477–490.

Appendix of Specimen Citations

Seeds were obtained from the following specimens:

Petunia integrifolia (Hook.) Schinz & Thell. subsp. **integrifolia** (Hook.) Wijsman var. **integrifolia**.

ARGENTINA. Entre Ríos: Dept. Concórdia: Parque San Carlos, 11 Dec. 1988, T. Ando & H. Kokubun A2 (SI, Ando).

Petunia integrifolia (Hook.) Schinz & Thell. subsp. **integrifolia** (Hook.) Wijsman var. **depauperata** (R. E. Fr.) L. B. Sm. & Downs.

BRAZIL. Santa Catarina: Mun. Garopaba: 7 Km S. of Campo Duna, 5 Nov. 1990, G. Hashimoto, T. Ando & K. Buto B224 (MBM, Ando, Hashimoto).

Petunia integrifolia (Hook.) Schinz & Thell. subsp. **inflata** (R. E. Fr.) Wijsman.

ARGENTINA. Misiones: Dept. Candelaria: Rt. P103, 1.6 km E. of the junction of route 4 and 103, 27°23'17"S, 55°34'00"W, 28 Nov. 1990, T. Ando & K. Buto A9 (MVFA, SI, Ando, Hashimoto).

Petunia integrifolia (Hook.) Schinz & Thell. subsp. **occidentalis** (R. E. Fr.) Wijsman.

ARGENTINA. Jujuy: Dept. Ledesma: Rt. 19, 16 km S.W. of the junction of route 34 and 19 to Higueritas, 23°56'56"S, 64°56'11"W, 14 Nov. 1991, T. Ando & S. Iida A103 (SI, Ando).

Petunia axillaris (Lam.) Britton, Sterns & Poggenb. subsp. **axillaris**.

URUGUAY. Dept. Montevideo: 0.3 Km E. of La Paz to Camino Uruguay, 34°45'48"S, 56°13'14"W, 16 Nov. 1988, T. Ando & H. Kokubun U1 (MVFA, Ando).

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ペチュニア品種の片親である *Petunia integrifolia* の4種内分類群（subsp. *integrifolia* var. *integrifolia*, subsp. *integrifolia* var. *depauperata*, subsp. *inflata*, subsp. *occidentalis*）を整理する目的で，*P. axillaris* を加えた全組み合わせの正逆交配を行い，着果率と得られた雑種の花粉稔性を調べた．花器に関する33形質と花色を雑種の指標とした．subsp. *occidentalis* を除く3種内分類群は互いに正逆とも高い着果率を示し，その雑種は高い花粉稔性を示した．subsp. *occidentalis* を父親とした交配はどれも着果せず，母親とした場合も着果率が劣り，得られた雑種個体数も少なく，その花粉稔性は低かった．subsp. *occidentalis* と他の種内分類群との雑種は，*P. axillaris* と他の種内分類群との雑種より花粉稔性が低いことから，subsp. *occidentalis* は他の種内分類群よりむしろP. axillarisに近縁と思われた．前報（Ando et al. 1995）の結果と総合して，subsp. *occidentalis* を独立種，*Petunia occidentalis* R. E. Fr. と結論した．

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